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THE MUTAGENIC POTENTIAL OF 4-NITROPHENYL ISOPROPYL (PHENYL) PHO--ETC(U)
JUL 81 L J SAUERS, F R PULLIAM, J T FRUIN
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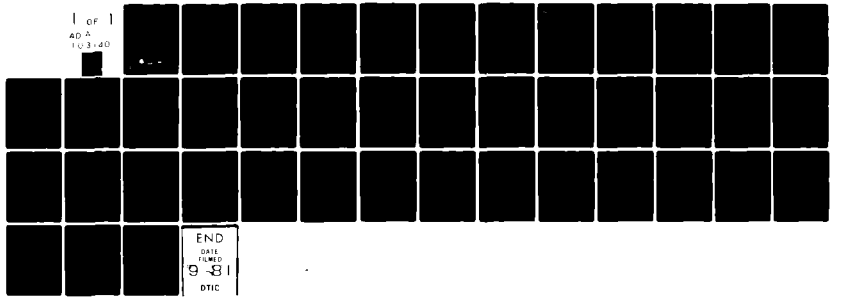


INSTITUTE REPORT NO. 100

THE MUTAGENIC POTENTIAL OF:

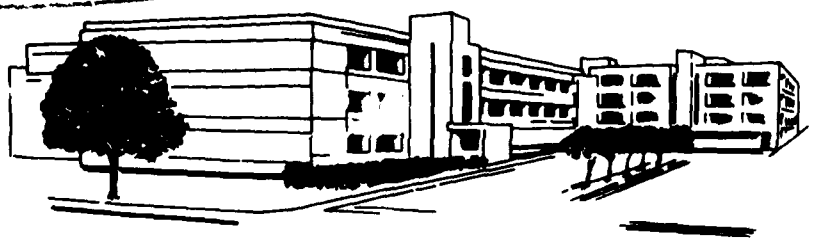
4-nitrophenyl isopropyl (phenyl) phosphinate
4-nitrophenyl ethyl (phenyl) phosphinate
phenyl 4-nitrophenyl (methyl) phosphinate
4-nitrophenyl 2-methoxphenyl phosphinate
4-nitrophenyl 4-nitrophenyl (methyl) phosphinate

LEONARD J. SAUERS, BA, SP5
FREDDICA R. PULLIAM, BS, SSG
and
JOHN T. FRUIN, DVM, PhD, LTC VC

TOXICOLOGY SERVICES GROUP,
DIVISION OF RESEARCH SUPPORT

JUL 1981

Toxicology Series: 4



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

Toxicology Series: 4

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of 4-nitrophenyl isopropyl(phenyl)phosphinate (103B); 4-nitrophenyl ethyl(phenyl)phosphinate (113); phenyl 4-nitrophenyl(methyl) phosphinate (103A); 4-nitrophenyl 2-methoxyphenyl(methyl)phosphinate (36); and 4-nitrophenyl 4-nitrophenyl(methyl)phosphinate (21) was assessed by using the Ames Salmonella/Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2×10^{-4} mg/plate. It was determined that none of the tested substances had mutagenic potential.

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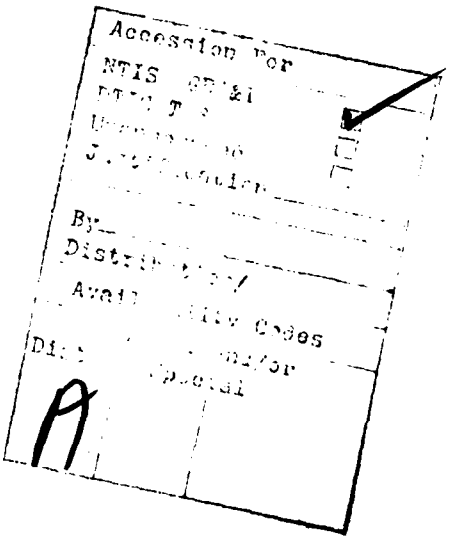
ABSTRACT

The mutagenic potential of 4-nitrophenyl isopropyl(phenyl) phosphinate (103B); 4-nitrophenyl ethyl(phenyl)phosphinate (113); phenyl 4-nitrophenyl(methyl)phosphinate (103A); 4-nitrophenyl 2-methoxy- phenyl(methyl)phosphinate (36); and 4-nitrophenyl 4-nitrophenyl (methyl)phosphinate (21) was assessed by using the Ames Salmonella/ Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2 x 10-4 mg/plate. It was determined that none of the tested substances had mutagenic potential.



PREFACE

AMES ASSAY REPORT:

SUBSTANCE	CODE NO.
4 nitrophenyl isopropyl(phenyl)phosphinate	103B
4 nitrophenyl ethyl(phenyl)phosphinate	113
phenyl 4-nitrophenyl(methyl)phosphinate	103A
4-nitrophenyl 2-methoxyphenyl(methyl)phosphinate	36
4-nitrophenyl 4-nitrophenyl(methyl)phosphinate	21

TESTING FACILITY: Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: Biomedical Laboratory, Aberdeen Proving Grounds
Aberdeen, MD 21005

PROJECT: Toxicity Testing of Phosphinate Compounds - 3516277ZA875

GLP STUDY NUMBER: 81002

STUDY DIRECTOR: LTC John T. Fruin D.V.M.,PhD.

CO-PRINCIPAL INVESTIGATORS: SSG Freddica R. Pulliam, B.S.

SP5 Leonard J. Sauers, B.A.

RAW DATA: A copy of the final report, study protocol and retired SOPs will be maintained in the LAIR archives. Test substances were provided by sponsor. Chemical, analytical, stability, purity, etc. data are available from the sponsor.

PURPOSE: To determine the mutagenic potential of the above compounds using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were used.

ACKNOWLEDGMENTS

The authors wish to thank Mr. John Dacey; SP4 Larry Mullen, BS; Ms. Carolyn Lewis, MS; CPT Nelson Powers, PhD, MS; SP5 Michael Rusnak, BS; and Evelyn McGown, PhD; for their assistance in performing the research and for help in preparation of this report.

Signatures of Principal Scientists
Involved in the Study

We, the undersigned, believe the study, GLP number 81002, described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

FREDDICA R. PULLIAM, BS Date
SSG
Co-Investigator

JOHN T. FRUIN, DVM, PhD Date
LTC, VC
Study Director

LEONARD J. SAUERS, BA Date
SP5
Co-Investigator

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

3 June 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that LAIR GLP study #81002 was a routine Ames Assay inspected as a routine process rather than specifically by study. The time-period of this study is included in the April 1981 report to management and the study director.

JOHN L. SZMREK
MAJ, MS
Quality Assurance Officer

TABLE OF CONTENTS

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to *in vivo* tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of *Salmonella typhimurium*, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (history of the strains used, methods to monitor the integrity of the organisms, and data pertaining to current and historical controls and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an *ampicillin* resistant R factor. Strains deficient in this plasmid demonstrate a zone of growth inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California, Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data, to determine if deviations from the set trends have occurred.

We compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains were used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned more than a 1000- fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number

of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliability of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by two independent methods. Ames et al (2) assumed that a compound which caused twice the spontaneous reversion rate is mutagenic. Commoner (5), developed the MUTAR Ratio, which is stated in the following equation:

$$MUTAR = (E - C)/C_{AV}$$

Here, C is the number of spontaneous revertant colonies on control plates obtained on the same day and with the same treatment and strains. E is the number of revertants in response to the compound; C_{AV} is the number of spontaneous revertants on control plates calculated from historical records. The explanation of the results of this equation can be determined by the method of Commoner (5). This variation determines the probability of correctly classifying substances as carcinogens on the basis of their mutagenic activity. The E values were recorded by strain, with and without S-9. Values for C and C_{AV} were recorded separately.

We used the formula and logged all values for our permanent records.

RESULTS

Ames Assay data were collected on 2, 8, and 11 March 1981. Throughout this report, all the test substances will be referred to by their respective code numbers.

Substance	Code No.
4-nitrophenyl isopropyl(phenyl)phosphinate	103B
4-nitrophenyl ethyl(phenyl)phosphinate	113
phenyl 4-nitrophenyl(methyl)phosphinate	103A
4-nitrophenyl 2-methoxyphenyl(methyl)phosphinate	36
4-nitrophenyl 4-nitrophenyl(methyl)phosphinate	21

The Toxicity Level Determination was run on 2 March 1981, for all the test compounds. All sterility, positive, and strain verification controls were normal. The spontaneous reversion rate

was below normal for nonactivated TA 100 (Table 1), the dosage spanned from 1 mg/plate to 1 x 10^{-7} mg/plate. In all instances, no toxicity was observed (Table 2A-E). It was decided to use 1 mg/plate as our initial dilution.

Two assays were conducted to determine the mutagenic potential of the five test substances. On 8 March 1981, the Ames Test was run on test compounds 103A, 103B, and 113. On 11 March 1981, substances 36 and 21 were assayed. The strain verification controls for the initial assay showed expected results in all instances (Table 3A). The spontaneous reversion rates were, for some strains, lower than suggested by Ames et al (2) but were within normal limits when compared to our historical data for all strains except TA 100. The spontaneous reversion rate for TA 100 was below our historical data base, both with and without S-9 (Table 3A).

In the second assay, we experienced unexpected results for TA 98, TA 100, and 1538 to UV light (Table 3B). We suspected mechanical problems, so this strain verification was retested on 14 March 1981. Expected results were obtained at this time. The lawns were uneven for all plates containing strain TA 1538. Since TA 98 and TA 1538 are alike in all aspects except for the addition of a plasmid in TA 98, we can disregard the data obtained for TA 1538 and still draw valid conclusions (Table 3B). The spontaneous reversion rates were within the range of our historical data for all strains except activated and nonactivated TA 100. Values for TA 100 were less than expected.

Unexpected reversion rates were seen in response to positive control chemical dimethyl benz-anthracene (DMBA) for all strains in the assay of 8 March 1981 (Table 4A). Although the tester strains lacked a high incidence of reversion in response to DMBA, they did respond to amino flourene (AF) and benz(α)pyrene (BP). These three chemicals function through the same mechanism. In the second assay, normal results were seen in response to all positive control chemicals except DMBA. TA 98, TA 100, TA 1537, TA 1538 showed below normal values (Table 4B).

DISCUSSION

The data relevant to the test-compound-induced spontaneous reversion rates are shown in Tables 5A-5E. For test substance 103A, a more than doubling of the spontaneous reversion rate is seen only for nonactivated TA 1537 at the 8 x 10^{-3} mg/plate dose. No dose response was seen (Table 5A).

For compound 103B, a more than doubling of the spontaneous reversion rate was seen for nonactivated TA 1537 at the 4 x 10^{-2}

mg/plate level. No dose response was observed (Table 5B).

Compound 113 shows a numerical suggestion of mutagenicity for nonactivated TA 1537 at the 1.6×10^{-3} mg/plate level. No dose response was seen (Table 5C).

The spontaneous reversion rate for TA 1537 determined on 8 March 1981, was low normal for the strain. It is the opinion of the Ames Assay Laboratory at the University of California, Berkeley, that even though a doubling of the revertant rate occurred, one cannot declare mutagenicity unless an obvious dose response is seen (Maron D., Ames Assay Laboratory, University of California, Berkeley, 30 March 1981). Although TA 1537 demonstrated some isolated incidences of a doubling of the spontaneous reversion rate, TA 100, the more sensitive strain, did not.

The Assay of 11 March 1981 showed a more than doubling of the spontaneous reversion rate for nonactivated TA 1535 at the 1 mg/plate dose for compound 36. No evidence of mutagenic activity is seen for compound 21. The data for TA 1538 was disregarded for these two test substances because uneven lawns were obtained (Tables 5D-5E). Our MUTAR values were well below the 1.5 threshold level in all instances (Tables 6A-6E).

CONCLUSION

For a substance to be mutagenic by the Ames Assay, several criteria must be met. We must see a doubling of the spontaneous reversion rate, a MUTAR value greater than 1.5, and an obvious dose response. In our assays a doubling of the spontaneous reversion rate occurred in only three isolated incidences and no dose response was observed. Therefore, we can conclude that test substances 103A, 103B, 113, 36, and 21 are not mutagenic.

RECOMMENDATION

We recommend that organo-phosphinate compounds 37, 73A, 83, 55, and 91 be tested using other toxicological testing systems if efficacy tests show those chemicals to be promising antidotes.

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LIST OF TABLES

TABLE I

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Strain No.	Histidine Requirements	Ampicillin Resistance	uvr-B Deletion	rfa Crystal Violet	Sterility Control	Response
TA 100	NG	9.45 mm	NG*	17.12 mm	G	+
TA 1537	NA	> 25 mm	NA	NA	NT	+
WT	G	NA	G	NA	NA	+
Diluent	NA	NA	NA	NA	NG	+
His Bio Mix	NG	MGA Plate	NG	Top Agar	NG	
Test Compound (s)		Diluent	NG			
(a) NG 103A	NA	NA	NA	NA	NG	+
(b) 103B	NA	NA	NA	NA	NG	+
(c) 113	NA	NA	NA	NA	NG	+
(d) 21	NA	NA	NA	NA	NG	+
(e) 36	NA	NA	NA	NA	NG	+

*Small number of colonies present

G = Growth; NG = No Growth; NT = Not Tested; NA = Not Applicable;
WT = Wild Type + = Expected Response

Spontaneous Revertants

Strain	TIME	S-9							AVERAGE
TA 100	End	No	80	51	56	42	70	68	61
	Start	No	53	47	50	65	65	74	59

Test Inculated By: Sauers, Pulliam, Dacey, McGown Date 2 March 1981

Test Read By: Len Sauers Date 4 March 1981

TABLE 2A

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) #103A (2) _____

(3) _____ (4) _____ (5) _____

Date: 2 March 1981 Performed by: Dacey, Pulliam, McGown, Sauers

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Code: 103A

Visual estimation of backgrourd lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1 mg/plate	64	61	71	65	NL
0.1	61	64	46	57	NL
0.01	53	67	56	59	NL
0.001	83	69	63	72	NL
0.0001	83	58	67	69	NL
0.00001	64	74	68	69	NL
0.000001	80	66	70	72	NL
0.0000001	64	63	55	61	NL

TABLE 2B

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) #103B (2) _____

(3) _____ (4) _____ (5) _____

Date: 2 March 1981 Performed by: Sauers, Pulliam, Dacey, McGown

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Code: 103B

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1 mg/plate	58	60	53	57	NL
10^{-1}	56	43	51	50	NL
10^{-2}	49	38	56	48	NL
10^{-3}	50	56	49	52	NL
10^{-4}	55	48	57	53	NL
10^{-5}	63	57	54	58	NL
10^{-6}	46	49	62	52	NL
10^{-7}	51	42	56	50	NL

TABLE 2C

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) #113 (2) _____

(3) _____ (4) _____ (5) _____

Date: 2 March 1981 Performed by: Sauers, Dacey, Pulliam, McGown

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Code: 113

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1 mg/plate	61	51	56	56	NL
10^{-1}	58	73	61	64	NL
10^{-2}	53	63	56	57	NL
10^{-3}	46	54	54	51	NL
10^{-4}	46	56	45	49	NL
10^{-5}	62	51	66	60	NL
10^{-6}	47	51	Contamination	49	NL
10^{-7}	56	61	43	53	NL

Table 2D

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed. (1) #36 (2) _____

(3) _____ (4) _____ (5) _____

Date: 2 March 1981 Performed by: Dacey, Pulliam, McGown, Sauers

Substance dissolved in: (1) DMSO (2) _____ (3) _____

(4) _____ (5) _____

Code: 36

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
SL = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1 mg/plate	75	102	94	90	NL
10^{-1}	63	65	50	59	NL
10^{-2}	75	99	73	82	NL
10^{-3}	63	65	55	61	NL
10^{-4}	83	85	98	89	NL
10^{-5}	54	63	73	63	NL
10^{-6}	64	83	79	75	NL
10^{-7}	65	49	63	59	NL

TABLE 2E

TOXICITY LEVEL DETERMINATION
Salmonella/Microsome Assay

Substance assayed: (1) #21 (2) _____

(3) _____ (4) _____ (5) _____

Date: 21 March 1981 Performed by: Sauers, Dacey, Pulliam, McGown

Substance dissolved in: (1) _____ (2) _____ (3) _____

(4) _____ (5) _____

Code: 21

Visual estimation of background lawn on Nutrient Agar Plates: NG = no growth
ST = slight growth
NL = normal growth

TA 100
Revertant Plate Count

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn
1 mg/plate	52	57	49	53	NL
10^{-1}	65	56	50	57	NL
10^{-2}	49	45	58	51	NL
10^{-3}	66	44	52	54	NL
10^{-4}	50	57	54	54	NL
10^{-5}	62	55	61	59	NL
10^{-6}	40	58	55	51	NL
10^{-7}	65	47	60	57	NL

TABLE 3A

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet	Sterility Control (e)
TA 98	+	+	+	18.41 mm	+
TA 100	+	+	+	19.92 mm	+
TA 1535	+	NA	+	18.83 mm	+
TA 1537	+	22.85 mm	+	18.21 mm	+
TA 1538	+	NA	+	19.11 mm	+
WT	Growth	NA	Growth	NA	Growth

QUALITY CONTROL (e)

His-Bio mix	Initial: +	End: +	Test Compound 1:	+	103A
Top Agar	Initial: +	End: +	Test Compound 2:	+	103B
S - 9	Initial: +	End: +	Test Compound 3:	+	113
Diluent:	+	Nutrient Broth: +	Test Compound 4:	NA	
MGA Plate w/ Bacteria:	+	MGA Plate: +	Test Compound 5:	NA	

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately [illegible]; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); WT not tested; NG=no growth; WT=wild type NA=not applicable.

Spontaneous Revertants

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
TA 98	40	30-50	20	21	19	17	21	18	20	20
TA 100	60	120-200	79	79	73	77	71	64	74	70
TA 1535	20	10-35	15	10	15	13	8	12	14	11
TA 1537	7	3-15	4	7	2	4	8	5	12	8
TA 1538	20	15-35	21	9	14	15	8	18	14	13

(1) Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: Sayers, Sullivan, Dacey, Mullen Date: 8 March 1981

Test Read By: Sayers Date: 10 March 1981

TABLE 3B

QUALITY CONTROL OF TESTER STRAINS WORKSHEET
Salmonella/Microsome Assay

Strain No.	Histidine (a) Requirements	Ampicillin (b) Resistance	uvr-B (c) Deletion	rfa Crystal Violet	Sterility Control (e)
TA 98	+	+	Growth*	13.42 mm	+
TA 100	+	+	Growth*	15.95 mm	+
TA 1535	+	NA	NG	18.80 mm	+
TA 1537	+	23.18 mm	NG	22.03 mm	+
TA 1538	+	NA	Growth*	21.95 mm	+
WT	Growth	NA	Growth	NT	Growth

QUALITY CONTROL (e)

His-Bio mix Initial: + End: + Test Compound 1: + 36

Top Agar Initial: + End: + Test Compound 2: + 21

S - 9 Initial: + End: + Test Compound 3: _____

Diluent: + Nutrient Broth: + Test Compound 4: _____

MGA Plate w/ bacteria: Growth MGA Plate: + Test Compound 5: _____

(a) + = no growth (requires histidine for growth); (b) + = no zone of inhibition, - = zone of inhibition of approximately 16mm; (c) + = no growth on irradiated side of plate; (d) + = zone of inhibition approximately 14mm diameter; (e) + = no growth (growth indicates contamination); NT=not tested; NG=no growth; WT=wild type NA=not applicable.

Spontaneous Revertants

Strain (1)	Avg	Range	No S-9			Avg	S-9			Avg
TA 98	40	30-50	22	18	13		28	36	15	
			20	15	8	16	34	23	28	27
TA 100	160	120-200	89	110	98		92	102	72	
			92	103	77	95	107	92	95	93
TA 1535	20	10-35	6	11	12		12	11	7	
			19	15	12	12	18	16	14	13
TA 1537	7	3-15	2	6	5		4	8	4	
			11	3	4	5	8	5	9	6
TA 1538	25	15-35	11	9	5		16	17	13	
			10	5	8	**	16	15	17	[illegible]

Ames, B.N., J. McCann and E. Yamasaki. Mutat. Res. 31:347

Test Inoculated By: Sauers,Pulliam,Dacey,Mullen Date: 11 March 1981

Test Read By: Sauers Date: 13 March 1981

*Unexpected response to UV; redone 14 Mar 81; obtained expected results. ** Lawns uneven.

TABLE 4A

POSITIVE CONTROL REVERTANT RATE

Date	Strain	Spontaneous Rev		AF	MNNG	BP	DMBA	Response (a)	Init.
		S 9	No S-9	S-9	No S-9	S-9	S-9		
3Mar81	A98	20	17	480	NA	116	28	-*	
	A102	70	77	517	178	251	96	-*	
	A1535	11	13	NA	854[illegible]	NA	NA	+	
	A1537	8	4	NA	NA	[illegible]	11	-*	
	A1538	13	15	442	NA	52	12	-*	

(a) + = expected result, - = unexpected result (see discipline note)

*Unexpected response to DMBA, 3 March 1981.

TABLE 4B

POSITIVE CONTROL REVERTANT RATE

Date	Strain	Spontaneous Rev		AF	MMS	BP	2-AA	Re-sponse (a)	Init
		S-9	No S-9	S-9	No S-9	S-9	S-9		
11Mar81	TA98	27	16	2405	NA	77	30	-*	
	TA100	92	95	1538	3082	204	126	-*	
	TA1535	13	12	NA	5801	NA	NA	+	
	TA1537	6	5	NA	NA	25	11	-*	
	TA1538	16	8	2161	NA	58	16	-*	

(a) + = expected result, - = unexpected result (see discussion text)

*Unexpected response to DMBA, 11 March 1981.

TABLE 5A

SALMONELLA/MICROSOME ASSAY WORK SHEET
(POSITIVE CONTROLS/TEST COMPOUNDS)

Substance Assayed: (1) 103A (2) _____

(3) _____ (4) _____ (5) _____

Date: 8 March 1981 Performed By: Sauers, Pullium, Lewis, Mullen

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537C	1538	1538C
103A	1 mg/pl	11	18	40	43	6	4	6	3	5	13
		22	20	48	53	18	7	4	6	12	12
		17	25	51	48	13	12	6	11	11	10
	Av.	17	21	46	48	12	8	5	7	9	12
103A	0.2 mg/pl	20	18	66	57	16	9	4	8	16	14
		11	20	49	48	15	10	8	4	11	8
		11	21	64		13	5	9	9	14	11
	Av.	14	20	60	52	15	9	7	7	14	11
103A	0.04 mg/pl	10	18	58	37	7	6	4	4	6	18
		14	15	53	47	5	9	9	9	10	9
		12	14	52	43	7	10	8	6	9	5
	Av.	12	16	54	42	6	8	7	6	8	11
103A	0.008 mg/pl	18	25	56	63	6	8	5	5	9	17
		12	22	32	65	7	5	13	7	18	22
		17	31	55	53	15	10	11	3	12	12
	Av	16	26	48	60	9	8	10	5	13	17
103A	0.0016 mg pl	14	22	67	58	22	7	5	5	3	8
		18	29	80	58	8	11	7	6	6	19
		19	28	56	63	13	7	4	4	5	9
	Av.	17	26	68	60	16	8	5	5	5	12

TABLE 5A

SALMONELLA/ MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Continuation Page

Revertant/Plate

Sub	conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
103A	0.00032 mg/pl	28	14	69	57	15	12	12	5	10	4
		11	23	59	58	19	11	5	12	10	4
		23		60	53	21	8	6	10	12	5
	Av.	21	18	63	56	18	10	8	9	11	6

TABLE 5B

SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) 103B (2) _____

(3) _____ (4) _____ (5) _____

Date: 8 March 1981 Performed By: Sauers, Pulliam, Lewis, Mullen

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
103B	1 mg/pl	11	12	57	58	11	8	6	5	6	14
		14	18	52	55	13	15	7	5	14	12
		18	20	33	52	13	9	4	9	12	12
	Av.	14	17	47	55	12	11	4	6	11	13
103B	0.2 mg/pl	24	18	50	63	13	14	8	11	14	1
		13	14	56	46	10	8	4	12	8	7
		16	12	67	57	8	10	9	10	9	7
	Av.	18	15	58	55	10	11	7	11	10	5
103B	0.04 mg/pl	11	21	71	49	4	5	7	9	9	16
		13	20	56	58	5	9	8	4	10	8
		14	21	43	56	3	5	12	8	5	7
	Av.	13	21	57	54	4	6	9	7	8	10
103B	0.008 mg/pl	24	13	31	49	13	10	5	9	11	13
		18	23	61	70	20	10	8	7	9	9
		19	14	43	58	12	9	10	6	11	11
	Av.	20	17	45	59	15	10	8	7	10	11
103B	0.0016 mg/pl	17	18	63	37	11	14	10	7	10	7
		12	19	57	51	21	5	3	4	13	13
		20	24	40	43	16	7	7	3	13	12
	Av.	16	20	55	44	16	9	7	5	12	11

TABLE 5B

SALMONELLA/ MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Continuation Page

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
1038	0.00032 mg/pl	24	23	38	38	11	7	13	5	5	19
		14	24	56	58	5	8	4	3	9	9
		9	22	52	53	10	7	6	12	13	12
	Av.	16	23	49	50	9	7	8	7	9	13

TABLE 5C

SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) 113 (2) _____

(3) _____ (4) _____ (5) _____

Date: 8 March 1981 Performed By: Sauers, Pullium, Lewis, Mullen

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

		# Revertant/Plate									
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538A
113	1 mg/pl	25	9	31	81	7	7	6	5	3	13
		23	14	52	63	13	6	3	4	8	10
		13	12	47	72	12	9	5	6	9	14
	Av.	20	12	43	72	11	7	5	5	7	12
113	0.2 mg/pl	17	13	43	73	7	5	9	5	7	9
		16	16	42	62	6	9	4	7	8	10
		27	11	60	61	13	9	11	5	10	10
	Av.	20	13	48	65	9	8	8	6	8	10
113	0.04 mg/pl	21	13	66	52	18	18	6	6	15	5
		13	6	62	48	15	16	7	5	8	11
		13	20	68	64	17	17	9	3	10	15
	Av.	16	13	65	55	17	17	7	5	11	10
113	0.008 mg/pl	23	17	52	58	17	13	5	8	17	10
		16	17	51	68	11	14	8	4	18	16
		19	16	63	74	16	8	6	6	16	14
	Av.	19	17	55	67	15	12	6	6	17	13
113	0.0016 mg/pl	15	26	58	65	26	17	7	4	12	11
		9	14	54	72	23	14	8	3	13	6
		11	22	62	58	19	12	11	4	9	10
	Av.	12	21	58	65	23	14	9	4	11	9

TABLE 5C

SALMONELLA/ MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Continuation Page

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538	1538
113	0.00032 mg/pl	16	10	54	68	23	18	8	7	9	12
		12	24	56	71	16	20	6	8	13	9
		15	21	61	73	18	24	7	9	9	14
	Av.	14	18	57	71	19	21	7	8	10	12

TABLE 5D

SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) 36 (2) _____

(3) _____ (4) _____ (5) _____

Date: 11 March 1981 Performed By: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

		# Revertant/Plate									
Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538*	1538A*
36	1 mg/pl	21	24	82	103	31	17	6	8	15	20
		17	24	81	92	24	12	5	6	18	20
		22	23	91	96	19	12	7	5	8	13
	Av.	20	24	85	97	25	14	6	6	14	18
36	0.2 mg/pl	21	6	90	69	21	13	2	5	10	17
		15	0	84	50	21	10	7	3	5	13
		17	0	88	44	20	15	3	4	4	9
	Av.	18	2	87	54	21	13	4	4	6	13
36	0.04 mg/pl	16	21	62	96	14	8	8	5	12	15
		22	25	60	73	13	12	7	1	8	18
		18	23	81	74	18	8	6	2	9	19
	Av.	19	23	68	81	15	9	7	3	10	17
36	0.008 mg/pl	10	18	76	94	27	6	4	7	11	16
		9	21	72	76	19	12	2	3	12	19
		21	28	70	80	19	16	2	6	10	19
	Av.	13	22	73	83	22	11	3	5	11	21
36	0.0016 mg/p	10	21	92	71	18	6	8	3	3	15
		13	26	77	72	13	8	3	6	7	18
		12	23	85	74	9	9	contami-nation	5	4	16
	Av.	12	23	85	72	13	8	6	5	5	16

*Uneven lawns on all plates with TA1538 were observed.

TABLE 5D

SALMONELLA/ MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Continuation Page

Revertant/Plate

Sub	Conc	93	98A	100	100A	1535	1535A	1537	1537A	1538*	1538A*
36	0.00032 mg/pl	25	23	81	84	20	8	4	10	12	23
		16	26	70	89	20	7	5	5	7	18
		18	17	67	73	15	12	7	10	14	14
1	Av.	20	22	73	82	18	9	5	8	11	18

*Uneven lawns on all plates with TA1538 were observed.

TABLE 5L

SALMONELLA/MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Substance Assayed: (1) #21 (2) _____

(3) _____ (4) _____ (5) _____

Date: 11 March Performed By: Sauers, Pulliam, Dacey, Mullen

Substance dissolved in: (1) DMSO (2) _____

(3) _____ (4) _____ (5) _____

Revertant/Plate

Sub	Conc	98	98A	100	100A	1535	1535A	1537	1537A	1538*	1538A*
21	1 mg/pl	23	7	68	64	5	3	1	1	16	8
		40	8	58	59	6	9	6	4	22	3
		26	12	68	44	6	4	4	3	31	11
	Av.	30	9	65	56	6	5	4	3	23	7
21	0.2 mg/pl	24	19	51	86	3	11	2	6	21	32
		14	17	54	68	5	9	4	5	26	29
		5	19	59	91	8	6	3	5	32	32
	Av.	14	18	55	82	5	9	3	5	26	31
21	0.04 mg/pl	30	16	56	55	21	8	5	6	10	13
		19	24	79	66	13	10	2	9	23	16
		10	29	67	77	16	11	3	2	17	26
	Av.	20	23	67	66	17	10	3	6	17	18
21	0.008 mg/pl	14	15	76	96	21	9	2	5	6	20
		14	14	82	82	7	8	8	2	9	23
		10	14	70	75	11	7	7	3	9	15
	Av.	13	14	76	84	13	8	6	3	8	19
21	0.0016 mg/pl	13	12	86	77	9	15	4	3	3	19
		11	16	104	90	8	4	1	1	4	10
		17	10	73	65	9	9	3	6	11	18
	Av.	14	13	88	77	9	9	3	3	6	16

*Uneven lawns on all plates with TA1538.

TABLE 5E

SALMONELLA/ MICROSOME ASSAY WORKSHEET
(POSITIVE CONTROLS/TEST COMPOUND)

Continuation Page

Revertant/Plate

Sub	Conc	93	98A	100	100A	1535	1535A	1537	1537A	P*	1538*
21	0.00032 mg/pl	8	19	73	88	9	5	2	6	4	6
		15	20	61	85	14	7	7	5	7	15
		6	27	57	108	18	13	5	4	4	10
	Av.	10	22	64	94	14	8	5	5	5	10

*Uneven lawns on all plates with TA1538.

TABLE 6A

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: Code 103A Dissolved in: DMSO

Date: 23 March 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 mg/plate	TA98	*	0.04	0.008	TA1535	*	*
0.2	TA98	*	*	0.0016	TA1535	0.24	*
0.04	TA98	*	*	0.00032	TA1535	0.4	*
0.008	TA98	*	0.22				
0.0016	TA98	*	0.22	1 mg/plate	TA1537	0.16	*
0.00032	TA98	0.18	*	0.2	TA1537	0.49	*
				0.04	TA1537	0.49	*
1 mg/plate	TA100	*	*	0.008	TA1537	0.98	*
0.2	TA100	*	*	0.0016	TA1537	0.16	*
0.04	TA100	*	*	0.00032	TA1537	0.65	0.14
0.008	TA100	*	*				
0.0016	TA100	*	*	1 mg/plate	TA1538	*	*
0.00032	TA100	*	*	0.2	TA1538	*	*
				0.04	TA1538	*	*
1 mg/plate	TA1535	*	*	0.008	TA1538	*	0.24
0.2	TA1535	0.16	*	0.0016	TA1538	*	*
0.04	TA1535	*	*	0.00032	TA1538	*	*

*Calculated value resulted in a negative MUTAR.

TABLE 6B

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: Code 103B Dissolved in: DMSO

Date: 23 March 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 mg/plate	TA98	*	*	0.008	TA1535	0.16	*
0.2	TA98	0.05	*	0.0016	TA1535	0.24	*
0.04	TA98	*	0.04	0.00032	TA1535	*	*
0.008	TA98	0.14	*				
0.0016	TA98	*	*	1 mg/plate	TA1537	*	*
0.00032	TA98	*	0.11	0.2	TA1537	0.49	0.41
				0.04	TA1537	0.82	*
1 mg/plate	TA100	*	*	0.008	TA1537	0.65	*
0.2	TA100	*	*	0.0016	TA1537	0.49	*
0.04	TA100	*	*	0.00032	TA1537	0.65	*
0.008	TA100	*	*				
0.0016	TA100	*	*	1 mg/plate	TA1538	*	*
0.00032	TA100	*	*	0.2	TA1538	*	*
				0.04	TA1538	*	*
1 mg/plate	TA1535	*	*	0.008	TA1538	*	*
0.2	TA1535	*	*	0.0016	TA1538	*	*
0.04	TA1535	*	*	0.00032	TA1538	*	*

*Calculated value resulted in negative MUTAR.

TABLE 6C

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: Code 113 Dissolved in: DMSO

Date: 23 March 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 mg/plate	TA98	0.14	*	0.008	TA1535	0.12	0.1
0.2	TA98	0.14	*	0.0016	TA1535	0.79	0.3
0.04	TA98	*	*	0.00032	TA1535	0.48	1.0
0.008	TA98	0.09	*				
0.0016	TA98	*	0.04	1 mg/plate	TA1537	0.16	*
0.00032	TA98	*	*	0.2	TA1537	0.65	*
				0.04	TA1537	0.49	*
1 mg/plate	TA100	*	0.02	0.008	TA1537	0.33	*
0.2	TA100	*	*	0.0016	TA1537	0.82	*
0.04	TA100	*	*	0.00032	TA1537	0.49	*
0.008	TA100	*	*				
0.0016	TA100	*	*	1 mg/plate	TA1538	*	*
0.00032	TA100	*	.01	0.2	TA1538	*	*
				0.04	TA1538	*	*
1 mg/plate	TA1535	*	*	0.008	TA1538	0.24	*
0.2	TA1535	*	*	0.0016	TA1538	*	*
0.04	TA1535	0.32	0.5	0.00032	TA1538	*	*

*Calculated value resulted in negative MUTAR.

TABLE 6D

MUTAGENIC ACTIVITY RATIO
Salmonella/Microsome Assay

Substance Assayed: Code 36 Dissolved in: DMSO

Date: 23 March 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 mg/plate	TA98	0.18	*	0.008	TA1535	0.79	*
0.2	TA98	0.09	*	0.0016	TA1535	0.05	*
0.04	TA98	0.14	*	0.00032	TA1535	0.48	*
0.008	TA98	*	*				
0.0016	TA98	*	*	1 mg/plate	TA1537	0.16	*
0.00032	TA98	0.18	*	0.2	TA1537	*	*
				0.04	TA1537	0.33	*
1 mg/plate	TA100	*	0.03	0.008	TA1537	*	*
0.2	TA100	*	*	0.0016	TA1537	0.16	*
0.04	TA100	*	*	0.00032	TA1537	*	0.22
0.008	TA100	*	*				
0.0016	TA100	*	*				
0.00032	TA100	*	*				
1 mg/plate	TA1535	1.03	0.1				
0.2	TA1535	0.71	*				
0.04	TA1535	0.24	*				

*Calculated value resulted in negative MUTAR.

TABLE 6E

MUTAGENIC ACTIVITY RATIO
Salmonella Microsome Assay

Substance Assayed: Code 21 Dissolved in: DMSO

Date: 23 March 1981 Performed by: Sauers

Concentration	Strain	MUTAR	MUTAR act	Concentration	Strain	MUTAR	MUTAR act
1 mg/plate	TA98	0.65	*	0.008	TA1535	0.0[illegible]	*
0.2	TA98	*	*	0.0016	TA1535	*	*
0.04	TA98	0.18	*	0.00032	TA1535	0.[illegible]	*
0.008	TA98	*	*				
0.0016	TA98	*	*	1 mg/plate	TA1537	*	*
0.00032	TA98	*	*	0.2	TA1537	*	
				0.04	TA1537	*	*
1 mg/plate	TA100	*	*	0.008	TA1537	0.16	*
0.2	TA100	*	*	0.0016	TA1537	*	*
0.04	TA100	*	*	0.00032	TA1537	*	*
0.008	TA100	*	*				
0.0016	TA100	*	*				
0.00032	TA100	*	0.01				
1 mg/plate	TA1535	*	*				
0.2	TA1535	*	*				
0.04	TA1535	0.[illegible]	*				

*Calculated value resulted in negative MUTAR.

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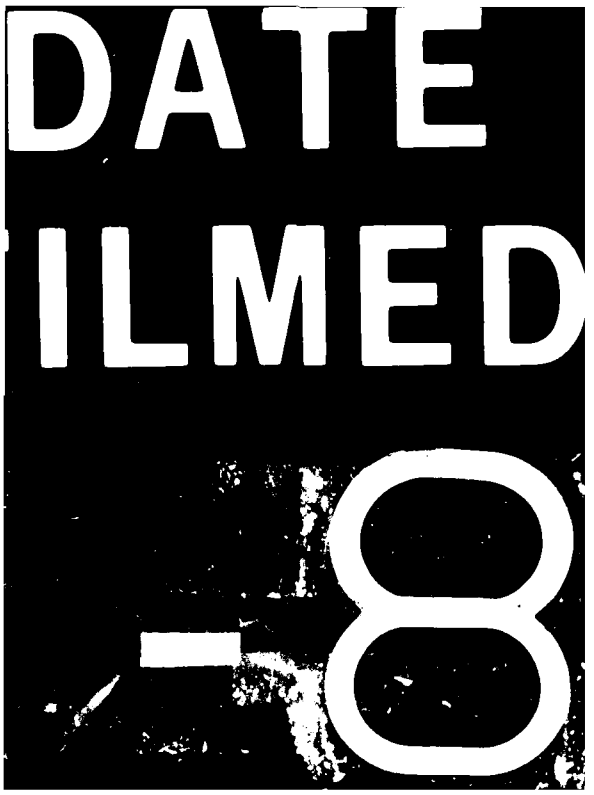
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